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Diabetes Alert Dogs: objective behaviours shown during periods of owner glucose fluctuation and stability

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Owner-independent assessments of Diabetes Alert Dog (DAD) behaviour post-placement are currently lacking. Here we describe the first study to simultaneously collect objective DAD behavioural data from CCTV footage and concurrent owner glucose levels via a Flash Glucose Monitoring System (FGMS). Using a pre-defined behavioural ethogram, both trained and non-trained canine behaviours were recorded. Given that dogs are trained to display attention-seeking behaviours when their owners experience fluctuations outside of normal blood glucose levels, we would expect differences in DAD behaviour during periods when owner glucose levels transition from euglycaemia to hyperglycaemic (high) or hypoglycaemic (low) levels, as compared to periods when owners stay within target-range.

A FreeStyle Libre FGMS was given to nine owners of accredited DADs from a single training establishment. Behavioural data were collected using CCTV in the participants' home, or place of work, for between five and 14 days (*mean* = 12.2 days). For each person, between 19 and 29 (*mean* = 22.5) one-hour periods were selected that captured approximately equal instances of owner glucose levels transitioning from in-range to hypoglycaemia, in-range to hyperglycaemia, or remaining within target-range. Two researchers coded footage without knowledge of the owner's glucose levels. Variables recorded included the DAD's activity, attentional state, proximity to owner, attention-seeking, and potential stress-related behaviours.

There were significant differences between individual dogs' behaviour during in-range periods. When samples captured a transition to out-of-range glucose levels (hypo- or hyperglycaemia), the distribution of several behaviours among dogs in the cohort differed significantly from their distributions during in-range samples ($p < 0.01$ for Conover Tests in *Playing with Owner*, *Jump Up*, *Sniff Owner*, *Bark*, *Paw Owner*, and *Lick Owner*), but consistent increases or decreases in the rate of any behaviours were not detected across the cohort. In individual dogs, we found distinctive behaviour changes during periods when their owner's glucose transitioned to out-of-range, as compared to when they remained in-range.

Each DAD showed significant changes in variance of at least one trained attention-seeking behaviour, with several dogs also showing changes in non-trained behaviours such as *Change of State*, *Playing with Owner* and potential stress-related behaviours (*Yawning* and *Lip-Licking*). This is the first study to objectively show that DADs differ in their behaviour during periods of owner glucose fluctuation and further highlights the individuality of responses. Understanding this variation, and factors affecting it, is fundamental to optimising DAD performance.

Key words: diabetes, alert, hyperglycaemia, hypoglycaemia, olfaction, canine

1. Introduction

While domestic dogs have traditionally been used for functions such as hunting and herding, more recently their roles have expanded to include conservation, military, law enforcement, and a range of medical support and disease detection tasks (Rooney, 2016). Dogs have successfully been trained to detect various types of cancer (e.g. Pickel et al., 2004; Willis, 2004; Horvath et al., 2013; Taverna et al., 2015), and, more recently, out-of-range glucose levels in individuals with both Type 1 and 2 diabetes (e.g. Rooney et al., 2013; 2019; Hardin et al., 2015; Gonder-Frederick et al., 2017a; 2017b). Diabetes Alert Dogs (DADs) potentially offer a non-invasive method of assisting in the recognition of an oncoming hypo- or hyperglycaemic episode by alerting their owner whilst they are still able to act, thereby vastly improving quality of life (Rooney et al., 2013).

Initial published reports on DADs came from case study evidence and were limited to small numbers of un-trained dogs that appeared to spontaneously show behaviours immediately prior to, or during, their owner experiencing a hypoglycaemic episode (e.g. Chen et al., 2000, O'Connor, 2008). Subsequently, organisations started training dogs, and over the last decade their use has expanded, with organisations in several countries placing dogs trained to 'alert' their owner, using attention-seeking behaviours, when they detect their blood glucose levels have deviated from a specified target range. In recent years, proof-of-principle studies have confirmed that dogs can differentiate between *in vitro* hypoglycaemic and euglycaemic (in-range) perspiration and breath samples (Hardin et al., 2012; 2013; 2015). However, methods of training and testing have been shown to affect alerting accuracy (Dehlingher et al., 2014). Previous studies have also assessed the psychosocial impact of owning a DAD, finding significant benefits (e.g. Wells et al., 2008, Rooney et al., 2013). Until recently however, there was a lack of investigation into the accuracy of trained DADs once placed with an owner. Studies have now started to compare owner's reports of their DAD's alerts to their reported blood test results (e.g. Gonder-Frederick et al., 2013; Rooney et al., 2013; 2019), and, more recently, to blood glucose data obtained from a Glucose Monitoring System (either Continuous: CGMS, or Flash: FGMS) (e.g. Gonder-Frederick et al., 2017a; Los et al., 2017). However, there remained a lack of objective assessment of dogs' alerts as, until Wilson et al. (2019), all studies relied on owners' reports of when their DAD alerted them. This has the potential to introduce conscious and/or unconscious bias, and to neglect behaviours outside of a dog's trained 'alert'. Previous studies of the accuracy of alerting behaviour have shown considerable variation between dogs (e.g. Gonder-Frederick et al., 2017a, Rooney et al., 2019; Wilson et al., 2019) despite, in some cases, the dogs having undergone the same training protocol. It is possible, that during periods of glucose fluctuation, some dogs show subtle changes in behaviour as well as distinct alerting behaviours. We provide the first owner-independent record of in-situ DAD behaviour by objectively measuring both trained and non-trained DAD behaviours during periods of owner glucose fluctuation and stability.

Here we use CCTV footage in DAD owners' work or home environment for up to 14 days alongside FGMS glucose data. This footage was used in Wilson et al. (2019) to assess the accuracy of DAD alerts, where alerts were categorized on the basis of behaviours defined by the DAD's trainer. An 'alert' may constitute several behaviours in conjunction with one another (e.g. pawing and nuzzling), or may be based on a single specific behaviour (e.g. fetch blood testing kit). In the current study therefore, we do not measure 'alerts', but instead record objective behaviours during periods of owner glucose fluctuation and stability to investigate whether there are differences in specific behaviours exhibited by the dogs during these episodes. Blind coders assess the footage without knowledge of the owner's glucose levels to provide the first owner-independent assessment of DAD behaviour.

Since dogs are trained to show attention-gaining behaviours, we would predict a change in the occurrence of these at times of glucose fluctuation. However, these may be accompanied by changes in attention or proximity to the owner, and in activity levels. It is also possible that surrounding a period of owner glucose fluctuation we would see an increase in behaviours indicative of arousal or stress. Decision-making is known to be stressful and has been shown to result in behavioural changes in numerous species including humans (Rilling & Sanfey, 2011), gorillas (Suda-King et al., 2013) and chickens (Davies et al., 2014). Hence, making a decision on whether to alert their owner may result in increased arousal or stress for a DAD. Therefore, the behaviours measured in this study were categorised as: Activity, Attentional state, Proximity to owner, Attention-seeking and Stress-related behaviours.

We hypothesise that there will be measurable differences in DAD's behaviour during periods in which glucose fluctuates to dangerously high or low levels, as compared to when it remains within safe limits. Since previous studies in this area have found notable variation in performance between dogs (Gonder-Frederick et al., 2017a; 2017b; Rooney et al., 2019; Wilson et al., 2019), we sought to assess changes on the cohort, and individual dog, level.

This study has two aims:

1) To assess cohort-wide variation in frequency and duration of behaviours during samples when the owner remains in-range, as compared to experiencing a transition to out-of-range glucose levels (hypo- and hyperglycaemia).
2) To assess individual patterns of behavioural change when a DAD owner transitions to out-of-range glucose levels (hypo- and hyperglycaemia).

2. Materials and Methods

2.1 Ethical approval

The study protocol was approved by The University of Edinburgh Royal (Dick) School of Veterinary Sciences Human Ethics Research Committee and The University of Bristol Faculty Research Ethics Committee (UB/17/014). Participants were provided with an information sheet prior to taking part, signed an informed consent sheet, and were reminded of their right to withdraw from the study at any time without needing to provide an explanation.

2.2 Medical Detection Dogs

DADs were recruited from Medical Detection Dogs, the leading charity in the United Kingdom that trains medical alert and disease detection dogs, and the only charity accredited by Assistance Dogs UK to train DADs (Medical Detection Dogs, 2017). Medical Detection Dogs initially train using *in vitro* samples obtained from the dog's prospective owner, progressing to *in vivo* training with that person. Hypoglycaemia samples are paired with a reward to shape the dog's behaviour in response to the olfactory cue. Dogs are trained to respond with specific behaviours, such as pawing their owner or fetching their blood testing kit, however additional attention-seeking behaviours may develop during training (Rooney, 2016). Since the primary aim is to train dogs to alert to hypoglycaemic episodes, which can be life threatening, this constitutes the majority of their training. However, some dogs spontaneously learn to respond to hyperglycaemic episodes and the charity rewards dogs when this occurs.

2.3 Participant recruitment

Recipients of a Medical Detection Dogs trained DAD were approached and recruited as described in Wilson et al. (2019). Of the eleven DAD owners approached, nine accepted: eight females and one male, aged 26 to 63 (*median* = 52.2 years). Dogs were seven neutered males and two spayed females; one Miniature Poodle, four Labrador Retrievers, one Golden Retriever and three Labrador-Golden Retriever crosses. All partnerships had been accredited for between two months and 73 months (*median* = 41.1 months). When giving consent, participants were requested to continue their pre-existing diabetes management plan without alteration.

2.4 Data collection

Participants were loaned a FreeStyle Libre Flash Glucose Monitoring sensor and scanner (Abbott Diabetes Care, Almeda, CA) to be worn for up to 14 days (after which sensors expire). Opaque plastic adhesive squares were used to cover the scanner screen and occlude glucose level results from the participant's view. Total time wearing the sensor ranged from five to 14 days per participant (*mean* = 12.2 days).

Video footage was obtained using a Swann CCTV System with up to four cameras assembled in the participant's home or place of work and stored on a Swann Digital Video Recorder (DVR). For the participant who was recorded at work, cameras were positioned so that only the participant and DAD (but not their colleagues) were in view.

2.5 Sample selection

Each participant had an individual target blood glucose range as determined by a medical practitioner (Table 1). Each dog had been trained to alert their owner when glucose levels deviated from this specific range, and they are rewarded on the basis of these glucose values. Every FGMS reading was categorized as 'in-range', 'hypo' or 'hyper', dependent upon the individual owner's target range. FGMS data were used by researcher NJR to select specific one-hour windows (samples), within which all pre-defined DAD behaviors were coded from CCTV footage. The FGMS device provides interstitial glucose levels at 15-minute intervals, with additional data every time the user scans the sensor. Selected sample hours captured either:

1. Stable period (where glucose levels remained in-range throughout).
2. Descending period (where glucose levels changed from in-range to hypoglycaemic – 'hypo').
3. Ascending period (where glucose levels changed from in-range to hyperglycaemic – 'hyper').

Hour samples were chosen on the basis that they consisted of four automatic data points obtained by the FGMS (at 15-minute intervals; and in some cases, additional scanned samples), and concurrent CCTV footage where the owner and DAD were in view of the cameras. Since dogs are reported to 'pre-alert' (see Rooney et al., 2019), all samples had at least two in-range recordings prior to their start, and, for hypo- and hyperglycaemic samples, the time at which the transition occurred varied between 15 and 45 minutes from the start of the sample. To prevent the blind-coder anticipating alert behaviours, the number of minutes within the 15 to 45-minute window that a glucose transition occurred was randomly generated. While the samples captured either euglycaemia or a *transition* to hypo- or hyperglycaemia, for brevity in this paper these sample types will be referred to simply as 'in-range', 'hypo' or 'hyper'.

The initial intention was to analyse an equal number of in-range, hypo and hyper samples for each participant, however this was not possible due to the nature of owners' fluctuating glucose levels, and how much time some individuals spent in view of the cameras. The total number of one-hour samples analysed ranged from 18 to 29 per participant (*mean* = 22.6 hours, Table 1).

2.6 Video coding

Data files obtained from the Swann DVR were combined using Windows Movie Maker to create hour-long video clips covering the sample window. Video samples were coded by either researcher CW or SK using The Observer XT Version 11.5. The researchers trained together to ensure consistent recording. FGMS values were not available to the blind coders at the point of coding to avoid bias. Since analysis was carried out within dog; to ensure consistency, a single researcher coded all the sample videos for a given participant; SK coded two partnerships and CW seven. Behaviours were coded from a pre-defined ethogram developed from the previously relevant literature (Table 2).

Behavioural variables fell into five categories: 1) Activity, 2) Attentional state, 3) Proximity to owner, 4) Attention-seeking and 5) Stress-related. Attention-seeking behaviour included trained alerting behaviours (e.g. *Fetch Blood Testing Kit*), but also likely spontaneous attention-seeking behaviours (non-trained; e.g. *Bark*). Potential stress-related behaviours (e.g. *Lip-Licking*) were included as defined by research by Beerda et al. (1998), Frederickson-MacNamara and Butler (2006) and Loftus et al. (2012).

In total, 200 hours of footage were analysed using The Observer XT 11.5. Data were extracted to Windows Excel. For 'state' behaviours, duration and frequency were extracted for each variable. For 'count' behaviours, only frequency was recorded (e.g. *Bark*) (see Table 2). A Spearman Rank Correlation Test identified pairs of variables within a single behaviour (duration and frequency) which were highly correlated (at Rho > 0.7). For each pair, one was eliminated and only the most biologically relevant retained; ten were thereby rejected. This resulted in 21 variables being retained for further analysis (Table 2). Inter-variable comparisons revealed that locomotion and standing were highly correlated (at Rho > 0.7) and thus were combined for analysis. The frequency of changes between activity states were calculated and labelled '*change of state*'.

2.7 Statistical analyses

Duration variables were highly skewed and no transformation was found to normalise them. Attempts to model frequency data as Poisson variables revealed substantial over-dispersion both between dogs and between time samples for the same dog. Therefore, non-parametric methods were used to analyse both types of data: Mann Whitney tests to assess shifts in the location of the distributions, and Conover's squared rank test to assess changes in their shape (Conover, 1999). We analysed the cohort as a whole and then explored partnerships individually.

For all analyses the duration of each activity was expressed as a percentage of the time in sight. Counts were expressed as rates per hour in view for each sample. This occasionally resulted in elevated event rates for behaviours that would be expected to occur only once in an out-of-range (OOR) episode (e.g. *Fetching Blood Testing Kit*). However, since both the Mann Whitney and the Conover Tests use ranks rather than absolute values, this is unlikely to distort the test results. We regard these analyses as exploratory, screening a large number of measures for their potential use in future studies. To compensate for multiple testing, a threshold of $p < 0.01$ was used to identify variables of interest when pooling information across dogs, and when screening for changes in the behaviour of individual dogs (for which there are more tests) a threshold of $p < 0.001$ was used.

SAS version 9.4 was used for all statistical analyses.

3. Results

3.1 Cohort tests

When the owner's glucose remained within target range, there were marked differences in all behaviours between dogs: *Yawning* ($p = 0.0045$), *Lying Head Down* ($p = 0.0005$) and $p < 0.0001$ for all other measures (Conover Tests, see Supplementary Material).

No consistent cohort-wide differences from these discrepant baselines were found in the average rates or durations of any behaviour using a threshold of $p < 0.01$ for the Mann-Whitney Test. However, six variables (*Playing with Owner*, *Jump Up*, *Sniff Owner*, *Bark*, *Paw Owner*, and *Lick Owner)* showed significant differences in dispersion between dogs during out-of-range (either hypo- or hyperglycaemia), as compared to in-range samples (Conover Tests, Table 3). *Playing with Owner* had a wider distribution in OOR (hyper and hypo) samples as compared to in-range ($p < 0.001$). *Jump Up* was more widely distributed in hyperglycaemic samples ($p < 0.001$). *Sniff Owner* was more widely distributed in hypoglycaemic samples ($p = 0.002$). *Bark* showed reduced variation in all OOR conditions (p

< 0.001), whilst *Paw Owner* had a greater spread of variance in OOR conditions ($p = 0.01$) and *Lick Owner* had a reduced variance in hyperglycaemic samples ($p = 0.007$) (however, with only six events in total from hyper and euglycaemic samples, this may be a spurious result). All cohort wide significant variables fell within either the Activity or Attention-Seeking categories. Since no Mann-Whitney Test result was significant at $p < 0.01$, only Conover Test results are presented (Table 3).

3.2 Individual dog tests

When analysing the data from each dog separately, we saw that individual dogs showed distinctive arrays of behavioural variation during OOR (hypo and hyper combined), hypo-, and hyperglycaemic samples. In this analysis also, no Mann-Whitney test result was significant at $p < 0.001$ and thus only Conover Test results are presented (Table 4).

Table 4 shows that, for many behaviours, dogs showed differences in variability between either OOR (hypo- and hyperglycaemia combined), hypo- or hyperglycaemic samples and euglycaemic samples. In total, dogs showed significant differences in distribution for between two and eleven variables each. Overall, all 22 behaviours measured showed a significant difference in at least one dog. All nine dogs showed differences in variables relating to Activity and Attention-Seeking, four showed variation in Proximity to Owner, three showed variation in Attentional State and three in Stress-related behaviours. Most commonly the variation was increased when owners' blood glucose was OOR (hypo- and hyper samples combined) and there were more significant differences during owner glucose transitions to hypoglycaemia than to hyperglycaemia (Table 4).

Individual patterns of behaviours differed for each dog during an in-range, hypo- and hyperglycaemic episode. For example, Dog 3 showed distinct increases in *Sitting*, *Fetching Blood Testing Kit*, *Lip-Licking*, *Change of State* and remaining *1-4 Dog Lengths from Owner* during samples when its owner transitioned to hypo- and hyperglycaemic states (Figure 1).

Dog 3 also exhibited *Playing with Owner, Nuzzle Owner, Paw Owner* and *Lick Owner* during hypo- and/or hyperglycaemic episodes but never during in-range episodes (Figure 1).

4. Discussion

All dogs showed significant changes in the variance of at least one behaviour during episodes when their owner transitioned to out-of-range glucose levels (Table 4). However, no two dogs showed the same profile of behaviour change. Previous studies (e.g. Gonder-Frederick et al., 2017b; Rooney et al., 2019) have highlighted the variation of alerting accuracy between DADs. The current study additionally demonstrates differences in the objective behaviours elicited, with each dog showing a distinct behavioural pattern in response to its owner's glucose levels. Our hypothesis that behaviours would differ during periods containing transitions to hypo- or hyperglycaemia (compared to remaining in-range) was supported, however it was the dispersion of these behaviors that differed, rather than the median levels. Furthermore, we saw significant differences between dogs during in-range periods, suggesting that, while owners were experiencing euglycaemia, each dog was showing an individual array of behaviours.

Variability between dogs, coupled with a small number of samples per dog, meant that cohort-wide differences in measures of central tendency were not detected. However, the use of Conover Tests allowed us to identify differences in the variation between conditions for multiple variables (Conover, 1999). As a cohort, only three behaviours were found to consistently change in out-of-range periods in general: *Playing with Owner* and *Pawing Owner* (increased in variation) and *Barking* (decreased in variation) (Table 3). In addition, *Jump Up* and *Sniff Owner* increased in variation in hyper- and hypoglycaemic samples respectively whilst *Lick Owner* was rare, but appeared to decrease in variation in hyperglycaemic samples. For most behaviours showing significant differences, there was an increase in variability in at least one of the OOR conditions. This generally resulted from several of the dogs showing an increased frequency or duration of the behaviour during OOR periods. Since the same trend was not seen in all dogs, the Mann Whitney test results remained non-significant whilst the Conover Test confirmed the change. Given the variability between dogs, these findings suggest that analyses on the individual dog level are likely to provide a more in-depth assessment of behaviour.

When considering individual DADs, we found that dogs showed distinct patterns of trained and non-trained behaviours during episodes of owner glucose fluctuation as compared to glucose stability. All nine dogs showed differences in variables relating to Activity and Attention-Seeking when their owner transitioned to hypo, hyper or both, suggesting that these behaviours may be most commonly viewed in association to an alert. In view of the amount of multiple testing already involved, direct comparisons of behaviours seen in hypo versus hyper episodes were not carried out. However, Table 4 demonstrates that for many behaviours we saw significant changes in either hypo or hyper episodes, but not in both. Formal testing in future, larger, studies may be able to address directly whether the type of glycaemic event the owner is experiencing may have an effect on the behaviours the dog elicits.

During out-of-range episodes, some behaviours occur more often in some dogs, but not all dogs respond in the same way, thus variability between dogs increases (Table 3). Although specific behaviours become more frequent in individual dogs, they are not always exhibited in all out-of-range samples, so there is increased variability (Table 4). As we reported in our earlier study of the same dogs (Wilson et al., 2019), the length of time since accreditation showed an inverse relationship with sensitivity of the DAD's alerts, so it is possible that length of time since accreditation contributes to the variability of behaviour. The challenge of future work is to understand why some dogs, on certain occasions, do not respond to out-of-range glucose levels. For example, do out-of-range episodes that deviate further from target range lead to more noticeable changes in behaviour?

During training, Medical Detection Dogs encourages DADs to develop their own instinctive alerts (as long as the behaviour is considered appropriate) and handlers reward accordingly. It is therefore unsurprising that results show individual combinations of behaviours elicited by each dog during episodes when its owner transitions to out-of-range. Within the category of attention-seeking behaviours, those seen to significantly vary during periods of owner glucose fluctuation will likely contribute to what an owner perceives as an 'alert'. A DAD's alert may constitute one behaviour (e.g. *Fetch Blood Testing Kit*), but other behaviours may accompany it. For example, Dog 3 (shown in Figure 1) shows the behaviour *Fetch Blood Testing Kit* in nine episodes when its owner experienced a fluctuation to out-of-range glucose levels (in comparison to no periods when its owner remained within glucose range), suggesting that this is Dog 3's 'alert' behaviour. However, we additionally saw an increase in the duration of *Sitting*, and the frequency of *Change of State* and *Lip-Licking* (Figure 1). This dog was seen to sit and wait while its owner tested their blood glucose levels after an alert, which may have contributed to the recorded increase of this behaviour. An increase in *Change of State* shows that this dog was moving between activity states (sitting, standing, locomotion) at a higher frequency when its owner's glucose levels were out-of-range as compared to in-range. This could result from the transitions of behaviour associated with carrying out a trained alert, but may also reflect a level of restlessness while the owner is out-of-range. An increase in *Lip-Licking* during out-of-range episodes potentially reflects arousal and is discussed below.

For all nine dogs, at least one non-trained behaviour varied during periods of owner glucose fluctuation. For five dogs, *Playing with Owner* was more variable in duration during OOR as compared to in-range glucose periods. This behaviour was coded when both the dog and owner were engaged in cooperative play, however either partner could initiate the play. Sometimes the dogs started the play session, but on several occasions, when a participant transitioned to out-of-range, we observed their dog approach them prior to performing a trained alert. An approach, in some instances, prompted the owner to interact with their dog, either by petting or by initiating play. DADs sometimes reciprocated the play before reinforcing their alert with additional attention-seeking behaviour. Such chains of behaviour demonstrate that, in some situations, there is ambiguity in the dog's alerting, or the owner does not correctly interpret their dog's behaviour. Dogs living as close companions, as well as working as trained assistance dogs, mean there are regular opportunities for owners to confuse affectionate attention-seeking with alert responses. It is possible that if dogs were all trained to show non-ambiguous alerting behaviours (outside of their normal repertoire), such as *Fetch Blood Testing Kit*, such miscommunication may be rarer. However, Medical Detection Dogs report increased success during training by utilising the dog's preferred alert, and even a dog trained to show a non-ambiguous alert behaviour may on occasion choose to approach its owner prior to carrying out an alert, for example to receive more olfactory information. Hence, the owner's ability to interpret both trained and non-trained behaviours is critical to appropriate responding and to rewarding and maintaining their dog's performance over time. Indeed, Wilson et al. (2019) showed that, for the cohort of the current study, those owners who responded to their DAD's alerts as described in the training protocol generally had the best performing dogs. Hence, we suggest that minimising ambiguity within this communication system, encouraging clear alert behaviour, and educating owners in reading subtle behaviour changes is integral to optimising team performance.

Three dogs showed a significant change in non-trained behaviours that have been associated with stress or arousal (*Lip-Licking* or *Yawning*) during periods when their owner's glucose transitioned out-of-range, and Figure 1 illustrates that the frequency of these behaviours was in some cases observably different, even if not shown universal enough to be significant at $p < 0.001$. Beerda et al. (1998) cite yawning as a behaviour that dogs may exhibit when experiencing moderate stress, and the rate of yawning has been used as a proxy measure of welfare (Rooney et al., 2007). Previous studies have used lip-licking as a measure of a dog's ability to cope in its social or physical environment and has similarly been used as a proxy welfare measure (e.g. Beerda et al., 1998; Rooney et al., 2007; Deldalle & Gaunet, 2014). However, some argue that lip-licking might be instead a spontaneous display of increased arousal or motivation (Miklósi et al., 2000). As all dogs received a food reward for a correct alert, it is also plausible that lip-licking was performed in anticipation of receiving this reward. Previous studies have shown that decision-making can cause arousal and displacement behaviours, and that the difficulty or complexity of the task can increase the frequency with which they are displayed (e.g. Leavens et al., 2001). The olfactory cue of hypo- or hyperglycaemic glucose levels is thought to trigger a DAD to carry out trained alert behaviours. How this olfactory cue is perceived by the dog, and the factors, both external (for example the strength of the cue) and internal (the dog's motivation to carry out the task) are not yet fully understood. It has been suggested that ambiguous tasks require more cognitive effort and are therefore more likely to result in behavioural indicators such as anxiety (Moffatt, 2005), and it has been shown that adding complexity to a task increases measures of stress in other mammals (e.g. Ventricelli et al., 2013). It is possible, therefore, that ambiguity of this scent (for example when an owner is nearer their glucose threshold for in-range) could influence the level of arousal experienced by the dog. We suggest that the relative arousal when glucose levels are close to euglycaemia as compared to considerably hypo- or hyperglycaemic may affect a dog's arousal and potential stress levels, and that studying this further may help our understanding of the training process. However, while we see an increase in range of stress-related behaviours for three dogs, we see an increase in variance of play behaviours for eight out of nine dogs. Play has been previously used as an indicator of good welfare (Boissy et al., 2007) and usually occurs when a dog is relaxed (Tuber et al., 1996). This increase in play may be explained through mis-communication of signals as described above, however nonetheless suggests that, for most of the dogs, out-of-range episodes were not inducing prohibitively high levels of stress.

The methods used in this study were novel and required statistical tests sufficiently sensitive to detect subtle changes in behaviour. We have attempted to control the number of false positive statistical tests reported by setting thresholds of $p < 0.01$ for across dog analyses and $p < 0.001$ for within dog analyses. Figure 1 illustrates that the data had very highly skewed distributions, often with a small number of non-zero observations in the out-of-range samples only. The Conover Test is more sensitive to these differences than the Mann-Whitney Test. Even with a threshold of $p < 0.001$, a single non-zero observation can result in a positive test result. It is also possible that aspects of our sampling strategy affected the results. We analysed full hours of behavioural footage. In some instances, the glucose shift to out-of-range occurred between 30-45 minutes into the sample, thus a substantial period of the sample occurred while the owner was in-range. The measured changes in behaviour may have been more pronounced over a shorter period. Using a smaller time sampling window (e.g. 30-minutes) around a glucose level fluctuation may allow for less behavioural 'noise' to be recorded and hence increase the chances of a significant difference in the Mann-Whitney Tests. This could provide a clearer picture of behavioural changes that may occur in response to owner glucose fluctuation. However, given the 15-minute intervals between glucose data points that the FreeStyle Libre FGMS provides, we deemed one hour more appropriate. In future, we suggest utilising a Continuous Glucose Monitoring Device and analysing more frequent glucose data points (e.g. every five minutes) in conjunction with smaller time windows surrounding a glucose transition.

Conclusions

This study provides the first objective evidence that trained dogs show behavioural responses to owner glucose levels. Notably, dogs respond to changes in their owner's glucose levels with individual combinations of both trained and non-trained behaviours. During periods when their owner experienced a transition from in-range to out-of-range blood glucose levels, all dogs showed an increase in the dispersion of at least one Attention-seeking and Activity behaviour. In general, there was an increase in these behaviours during out-of-range periods, but not sufficiently consistent to be detectable as an increase in the median rate. We additionally observed significant changes in the dispersion of non-trained behaviours such as *Lip-Licking* and *Playing with Owner*. This study draws attention to the range and complexity of behaviours shown by trained dogs during owner glucose fluctuation. Understanding the underlying causes behind the recorded variation in behaviour, in addition to how these behaviours are shaped over time, may underpin future strategies to optimise DAD function and performance.

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Author Contributions Statement

NJR developed the experimental design for this project and contacted the participants. CW created the materials used in this study. Researchers CW and NR were responsible for the transportation of equipment to participant's houses or places of work. CW assembled all participants' CCTV systems and administered the FreeStyle Libre subcutaneous sensors. NJR specified periods from the FGMS data for CW and SK to create video files of and code. SM carried out all statistical analyses. CG fed into the study design at numerous stages and commented on the draft manuscript. The manuscript was drafted by CW and NJR and commented on and edited by all authors.

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